Novel Research in Microbiology Journal (2019), 3(3): 379-386
Research Article

(Print) (ISSN 2537-0286)
(Online) (ISSN 2537-0294)
https://nrmj.journals.ekb.eg/
DOI: 10.21608/nrmj.2019.37211

Prevalence of keratinophiic fungi and other dermatophytes from soils of Nnewi in Anambra state, Nigeria

Eze, E.M.[1*]; Ezebialu, C.U.[2]; Unegbu, V.N.[3]; Nneji, I.R.[4]

[1]Department of Microbiology, Novena University, Ogume, Delta state, Nigeria; [2]Department of Microbiology, Godfrey Okoye University, Thinkers Corner Ugwumu-nike, Nigeria; [3]Department of Microbiology, Renaissance University, Ugboawka, Enugu State, Nigeria; [4]Department of Microbiology, Legacy University , Okija, Anambra State, Nigeria.

[*]Corresponding author E-mail: chuksebere31@yahoo.com

Received: 7 May, 2019; Accepted: 2 June, 2019; Published online: 25 June, 2019

Abstract

This study was carried out to isolate and identify the keratinophilic fungi and other dermatophytes present in soils of Otolo Nnewi, Nnewi north local government area, Anambra state, Nigeria. Eighty soil samples were collected from four habitats (playgrounds, abattoir, public parks, and poultry farms) of Otolo Nnewi; and were screened for the presence of keratinophilic fungi and dermatophytes, using hair baiting techniques for isolation of these fungi. Of which, 69 soil samples (86%) were positive for fungal growth. Fungal growth appearing on the baits after 2-4 weeks of incubation at 37°C were macroscopically and microscopically examined, and were also cultured on Sabroaud dextrose agar (SDA). These fungal isolates were identified on the basis of colony and microscopic features. A total of 150 isolates of keratinophiic fungi were detected. The isolated fungi were classified into 19 species belonging to 11 genera mainly; *Chrysosporium indicum*, *C. tropicum*, *Aspergillus flavus*, *Microsporum gypseum* and *Trichophyton terrestre* were isolated frequently. *C. indicum* (13%) was the most predominant isolated species; *C. tropicum* (12%) was the second, followed by *A. flavus* (11%). In the current study; *M. gypseum* (9.3%) was the most common isolated dermatophyte, followed by *T. terrestre* (6%) and *T. rubrum* (6%). *M. canis* was isolated only from soils of abattoir and public parks. Moreover, *C. zonatum*, *T. mentagrophytes*, *Alternaria alternata* and *Fusarium oxysporum* were also isolated. There was significant statistical difference ($p<0.05$) between the keratinophilic fungi and dermatophytes isolated from soil samples of these different habitats. Results obtained from this study indicated the wide occurrence of keratinophilic fungi in the studied area, which were potential agents of human and animals fungal diseases.

Keywords: Soil, Hair baiting, Keratinophilic fungi, Dermatophytes, Anambra state, Nigeria

1. Introduction

Keratinophytes are a group of fungi which colonize various keratinous substrates, and then degrade them to components of low molecular weight. These include a variety of mold fungi comprising mainly hyphomycetes. Hyphomycetes include dermatophytes and a variety of non-dermatophytic mold fungi (Mukesh and Sharma, 2010).

Soils that are rich in keratinous materials are most conducive for the growth and occurrence of these keratinophilic fungi. Their distribution differs with the environment and depends on different factors such as human and\ or animal presence. Keratinous substances occur in nature mainly in the form of; hairs, wools, feathers, horns, hooves, nails, skin, and other cornified appendages which constitutes natural baits for these fungi (Khanam and Jain, 2002). The majority of dermatophytes can live saprophytically, and every keratinophilic fungus can be considered as a potential pathogen. Marsella and Mercantini, (2016) pointed that dermatophytes cause human and animal mycoses, and thus have drawn the attention of medical and veterinary epidemiologists.

Keratinolytic fungi occur in many natural and man-made habitats. They exist in communities together with keratinophilic fungi that have weaker affinity to keratin, and utilize chiefly the products of its decomposition (Dominik and Majchrowicz, 2012). Based on their occurrence in natural habitats; keratinophilic fungi were divided into three categories: Anthropophilic, when human beings are the natural hosts; Zoophilic, when a variety of animals act as natural hosts; and Geophilic, when the soil is their natural habitat. Rizwana *et al*., (2012) reported that factors influencing the distribution of keratinophilic fungi have been well recognized in the soil environment. Keratinophilic microbes represent a huge biodiversity of forms, habitats, and substrates in the soil. It is therefore reasonable to anticipate soil as a huge reservoir of these keratinophilic fungi. Places like play grounds and public parks are often invaded by humans and animals. Soils which are contaminated with keratinaceous debris and propugules of fungal pathogens; cause infections in human beings and animals.

The current work reported the prevalence of keratinophilic fungi and its related dermatophytes in the soils of Nnewi, Anambra state, Nigeria. The aims of this work were to help us know the distribution and occurrence of keratinophilic fungi and other dermatophytes. In addition, it will also create awareness of the risk of human dermatophytosis in these areas.

2. Material and methods

2.1. Study area

This study was carried out in Otolo, Nnewi north local government area, Anambra state, Nigeria.

2.2. Collection of soil samples

A total of 80 soil samples were collected from different locations in Nnewi (i.e. Public parks, playgrounds, abattoir, and poultry farms). Soil samples were collected in sterile polyethylene bags by scooping up to a depth of 2-5 cm with the help of sterile disposable spoon. Each bag was tightly packed and labeled indicating the date and site of collection. These samples were brought to the laboratory, processed immediately and then stored at 4°C for further studies.

2.3. Isolation of the keratinophilic fungi

The keratinolytic fungi were isolated using Vanbreuseghem's hair bait technique (Vanbreuseghem, 1952). Sterile petri plates were half filled with soil samples. Short strands of sterilized human hair (0.6-1.6 cm in length) were spread over the surface of soil. About 10 to 15 ml of sterile water was added to the soil to facilitate the germination of the fungal conidia. Bacterial growth was prevented by adding chloramphenicol antibiotic (0.05 mg/ ml). Petri plates were incubated at room temperature for 2-4 weeks. Hair with fungus was aseptically picked from the petri plates, transferred to a test tube containing 5

ml sterile saline, and then shaken vigorously. The supernatant was carefully spread on SDA containing 0.05 mg/ ml chloramphenicol. One week after incubation; colonies growing on the medium were sub-cultured, and then pure cultures were stored on agar slants for identification.

2.4. Cultural characteristics of the fungal isolates

The different characteristics of the fungal colonies were examined. These included the color and texture of the colony on the surface of the SDA, nature and color on the reverse side of the slant, rate of growth, consistency, presence of pigments, nature and shape of fruiting bodies, and other peculiar features of the colony. These were used as guide to final identification of isolates.

2.5. Slide culture technique

Slide culture was prepared to examine and identify the fungi colonies in situ with as little disturbance as possible. Sterile filter paper was aseptically placed in petri dish with a pair of forceps and sterile V-shaped glass rod was placed on the filter paper. About 3 ml of sterile water was poured on the filter paper to completely moisten it. Using forceps, sterile slide was placed on the V-shaped rod. A scalpel was sterilised by flaming, and then gently used to cut a 5 mm square block from the plate of already prepared SDA. The agar block was aseptically transferred to the centre of the slide. The four sides of the agar square were inoculated with mycelia fragments of the fungus to be examined using sterile needle. Sterile cover slip was aseptically place on the upper surface of the agar cube. Then the petri dish was covered and incubated for 72 to 96 h. After incubation, a drop of lactophenol cotton blue stain was placed on a clean microscopic slide. The cover slip was removed from the slide culture and placed side down on the slide having the drop of lactophenol blue stain. In addition, the block of agar was discarded and a drop of lactophenol blue was added on the slide, and then covered with a cover slip. The two prepared slides were sealed with nail polish, and then examined under low power ×10 and high power ×40 objective lens, according to Haris, (1986).

2.6. Statistical analysis

All data collected were analyzed using SPSS 21 package, and level of significance was set at 0.05 as described earlier by Ogbeibu, (2005).

3. Results and Discussion

A Total of 80 soil samples were screened for the presence of dermatophytes and keratinophilic fungi. Of which, 69 soil samples (86%) were positive for keratinophilic and dermatophytes growth. It was observed that all soil samples collected from public parks were positive; whereas, the number of positive samples in play grounds, abattoir, and poultry farms were 18 (90%), 16 (80%) and 15 (75%), respectively (Table 1). 150 isolates, belonging to 11 genera and 19 species (Table 2), were isolated from various soils of Nnewi. Some of these soil samples however yielded mixed growth, while others presented single species.

Chrysosporium indicum, *C. tropicum*, *A. flavus*, *M. gypseum* and *Trichophyton terrestre* were isolated frequently. *C. indicum* (13%) was the most predominant species isolated. *C. tropicum* (12%) was the second, followed by *A. flavus* (11%). In the present study; *M. gypseum* (9.3%) was the most common isolated dermatophyte, followed by *T. terrestre* (6%) and *T. rubrum* (6%). However, *M. canis* was isolated only from soil samples of abattoir and public parks. *C. zonatum*, *T. mentagrophytes*, *A. alternata* and *F. oxysporum* were also isolated, besides other dermatophytes and keratinophilic fungi listed in Table (2). The playgrounds soils presented 31 isolates; abattoir (45 isolates), public parks (37 isolates) and poultry farms (37 isolates). These soils were found to be rich reservoirs of dermatophytes and keratinophilic fungi.

Table 1: Prevalence of keratinophilic fungi and dermatophytes isolated from soil samples of various habitats

Habitat	Playgrounds	Abattoir	Public park	Poultry farm	Total
No of samples studied	20	20	20	20	80
No of samples positive	18	16	20	15	69
Positive samples	90%*	80% *	100%*	75%*	86%

Where; *Values are not statically different (p>0.05)

Table 2: Frequency of occurrence of keratinophilic fungi and dermatophytes

Fungi Isolated	Playground	Abattoir	Public parks	Poultry farm	Total	% value
A. fulvescens	1	-	-	2	3	2%
C. indi*cum*	6	10	2	2	20*	13%
C. tropicum	3	1	4	10	18*	12%
C. zonatum	2	4	2	-	8	5%
M. gypseum	4	1	8	1	14*	9.3%
M. canis	-	-	2	5	7	5%
T. rubrum	1	5	3	-	9	6%
T. mentagrophytes	2	1	1	4	8	5.1%
T. terrestre	3	4	1	1	9	6%
A. niger	2	1	1	2	6	4%
A. flavus	1	8	3	4	16*	11%
A. terreus	-	2	3	4	9	6%
A. alternata	-	-	-	2	2	1.3%
F. oxysporum	-	1	2	-	3	2%
F. solani	2	2	2	-	6	4%
C. lunata	-	1	-	-	1	1%
P. chrysogenum	2	2	1	-	5	3.3%
R. stolonifer	1	2	-	-	3	2%
S. brevicaulis	1	-	2	-	3	2%
Total	**31**	**45**	**37**	**37**	**150**	

Where; *Values are statistically significant at p=0.05

This study clearly indicated the varied distribution of dermatophytes and keratinophilic fungi in soils of Nnewi. Keratinophilic fungi are important ecologically, and are present in the environment with variable distribution patterns which depend on different factors such as human and\ or animal presence. Diverse soil habitats have been screened from different countries for dermatophytes (Itisha and Kushwaha, 2010; Deshmukh and Veerkar, 2012), indicating that this group of fungi were distributed worldwide.

The most frequently isolated keratinophilic fungi in this study were *Chrysosporium indicum*, *C. tropicum*, *A. flavus*, *M. gypseum* and *T. terrestre*. The high prevalence of keratinophilic fungi in these soils demonstrated that hair of human, animals, and feather from birds which came to these soils either as dead and\ or dropped off, served as substrates and were subjected to microbial decomposition. Keratinophilic fungi played a significant role in the natural degradation of keratinized residues as reported by Sharma and Rajak, (2003). In a similar study; Itisha and Kushwaha, (2010) obtained 641 isolates from 125 soil samples of parks from Uttar Pradesh, India, indicating that soils of these parks were rich sources of many keratinophilic fungi and dermatophytes. Deshmukh and Agrawal, (2008) previously reported the isolation of *C. tropicum* and *C. indicum* from soils of Bahrain. Abdel and Zaki, (2008) isolated *C. indicum* from fields, animal and birds enclosures, animal hairs and birds feathers from Ismailia, Egypt. Ramesh and Hilda, (1999) also reported large number of keratinophilic fungi from primary schools and public parks of Madras city, India, indicating that these soils were reservoirs of diverse fungi.

Currently, *A. flavus* represented the third position of isolated fungi. It was previously reported as second dominant species in soils of Gorgan and Gonbad Kavus areas in Iran by Moallaei *et al*., (2006). Among the dermatophytes, *M. gypseum* was the most predominant fungus isolated from all the screened soils. This fungus was a common geophilic dermatophyte widely distributed globally in soils, and caused ringworm of the scalp and glabrous skin in human and animals (Ali and Rana, 2000). Singh *et al*., (2009) isolated *M. gypseum* from 13 hospital dust samples; it caused Tinea corporis and Tinea capitis in humans, and was also reported from cats, dogs and rodents.

The other isolated dermatophytes were *T. terrestre*, *T. mentagrophytes* and *T. rubrum* recorded in decreasing order. *T. mentagrophytes* was reported by Sahoo and Mahajan, (2016) as the causal agent of Tinea pedis, Tinea corporis, Tinea cruris and Onychomycosis. It has been isolated from soils of public parks in Mumbai, India (Sunil and Veerkar, 2012). Moreover, both *T. terrestre* and *T. mentagrophytes* has been recorded from hospital dust of Kanpur Uttar Pradesh (U.P), India, by Singh *et al*., (2009). *Chyrsoporium zonatum* and *A. alternata* accounted for the same prevalence in the present study. *Aphanoascus fulvescens* was isolated from garden soils. It has also been isolated from soils of Bahrain by Deshmukh and Agrawal, (2008).

Two *Fusarium* sp. were also isolated in this study. Simpanya and Baxter, (1996), recorded that *Fusarium* sp. were the most frequently isolated potential pathogens from soils collected from; parks, cleared areas, paddocks, river, and roadsides of New Zealand. *Scopulariopsis brevicaulis* was recovered from gardens, birds and animal enclosures. Ganaie *et al*., (2010); Mukesh and Sharma, (2010) reported *Scopulariopsis* sp., *Curvularia lunata* and *F. solani* from soils of gardens and school of Jhansi and Jaipur, India.

Fungi isolated in the current study were reported previously to be either well known agents of mycosis, or have been recovered from human and animal lesions especially for; *M. gypseum*, *T. rubrum*, *Geotricum candidum*, *A. flavus*, *F. oxysporum*, *Chrysosporium* sp. and others (Moallaei *et al*., 2006;

Sahoo and Mahajan, 2016). In general, dermatophytes are transmitted by contact with infected hairs, fomites (clippers, brushes), or from the environment (spores in soil). Sahoo and Mahajan, (2016) added that dogs and cats harbor many saprophytic molds and yeasts on their hair coats and skin. The most common isolates of these fungi were species of *Alternaria*, *Aspergillus*, *Cladosporium*, *Mucor*, *Penicillium*, and *Rhizopus* (Moallaei *et al*., 2006). Bernardo *et al*., (2005) pointed that most of these saprophytic isolates represented transient contaminants from soil, airborne, and were potential pathogens causing mycosis. Previous studies of Rizwana *et al*., (2012) reported that children playgrounds; animal inhabiting fields, and infected domestic animals constitute the main sources of infection for children, animal rearers and farmers, respectively. It is clear from current results that soils of parks, abattoir, poultry farms and playgrounds were ideal environments for the growth of keratinophilic fungi and dermatophytes. This could be attributed to the presence of significant organic debris and keratinous substrates such as; hairs, feathers from birds and animals, in addition to plant litters in these soils.

However, isolation of fungi was not uniform as it depends on organic matter. Organic matter content of soils was one of the major factors affecting the presence of keratinophilic fungi in these soils (Chmel *et al*., 2000). Identification of environments and fungi where people were exposed to was a major health concern (Madisen *et al*., 2007). Thus these human fungal pathogens could be considered as bio-indicators of environmental pollution with animal faeces, hairs, plant debris, and other keratinous substrates, and thus could pose risks of human and animal mycoses.

4. Conclusion

Keratin is one of the most abundant animal proteins on earth, as it forms a part of the exoskeleton of reptiles, birds and mammals. Among microbes that cycle this protein in nature, keratinophilic fungi are very common and the most diverse. During the course of evolution; many of the soil-associated keratinophilic fungi have adopted a pathogenic life cycle, and were potential agents of humans and animals fungal diseases. If these keratinophilic fungi were not there to cycle this highly stable protein (keratin); thus one can imagine the high quantity of keratin that would have accumulated on earth, as vast quantities of this protein are shed by the vertebrates. Accordingly, there is a need for further taxonomic and ecological studies of this interesting group of fungi.

Conflict of interests

The authors declare no conflict of interests.

Acknowledgements

The authors would like to thank the entire staff of Novena University, Ogume Delta state, Nigeria, who made this study possible. Also special thanks are to Mr\ Gideon Ogu, Biological science department, for his technical assistance during the course of this work.

5. References

Abdel, R.M. and Zaki, S.M. (2008). Experimental Pathogenicity and Molecular Characterization of an Environmental Isolate of *Chrysosporium zonatum* Al Musallam and Tan (Family: Onygonaceae, Order: Onygonales). International Journal of Agriculture and Biology. 10(3): 273-277.

Ali, Z.M. and Rama, M. (2000). Isolation of dermatophytes and related keratinophilic fungi from the two public parks in Ahvaz. Jundishapur Journal of Microbiology. 1(1): 20-23.

Bernardo, F.A.; Lança, M.M.; Guerra, H.I. and Marina, M. (2005). Dermatophytes isolated from pet, dogs and cats, in Lisbon, Portugal. RPCV.100 (553-554): 85-88.

Chmel, L.A.; Hasilikova, J.; Hrasko, U. and Vlacilikova, A. (2000). The influence of some ecological factors on keratinophilic fungi in the soil. Sabouraudia. 10(1): 26-34.

Deshmukh, S.K. and Veerkar, S.A. (2012). Keratinophilic fungi from selected soils of Bahrain Mycopathologia. 165(16):143-147.

Deshmukh, S.K. and Agrawal, S.C. (2008). Dermatophytes and keratinophilic fungi and their secondary metabolites. In: Kushwaha R.K.S. (ed.), Fungi in human and animal health. Jodhpur, India. pp. 85-111.

Dominik, T. and Majchrowicz, I. (2012). A trial for isolating keratinolytic and keratinophilic fungi from the soils of the cemeteries and forests of Szczecin. Ekologia Polska-Seria A. 12(5): 79-105.

Ganaie, M.A.; Sood, S.G.; Rizvi, M.J. and Khan, T.A. (2010). Isolation and Identification of Keratinophilic fungi from different soils samples in Jhansi City (India). Plant Pathology Journal. 9(4): 194-197.

Haris, J.L. (1986). Modified method for fungal slide culture. Journal of Clinical Microbiology. 24(3): 460-461.

Itisha, S. and Kushwaha, R.K. (2010). Dermatophytes and related Keratinophilic fungi in soil of parks and agricultural fields of Uttar Pradesh, (India). Indian Journal of Dermatology. 55(3): 306-308.

Khanam, S.J. and Jain, P.C. (2002). Isolation of keratin degrading fungi from soil of Damoh, India. Asian Journal of Microbiology, Biotechnology and Environmental Sciences. 4: 251-254.

Madisen, A.M.; Hansen, V.M.; Meyling, N.V. and Eilenberg, J. (2007). Human exposure to airborne fungi from genera used as biocontrol agents in plant protection. Annals of Agricultural and Environmental Medicine.14(4): 5-24.

Marsella, R. and Mercantini, R. (2016). Keratinophilic fungi isolated from soils of the Abruzzo National Park Italy. Mycopathologia. 94(23): 97-107.

Moallaei, H.F.; Zaini, M.; Pihet, M.; Mahmoudi, J. and Hashemi, N. (2006). Isolation of Keratinophilic Fungi from Soil Samples of Forests and Farm Yards. Iranian Journal of Public Health. 35(4): 62-69.

Mukesh, S. and Sharma, M. (2010). Incidence of dermatophytes and other keratinophilic fungi in the schools and college playground soils of Jaipur, (India). African Journal of Microbiology Research. 4(24): 2647-2654.

Ogbeibu, A.E. (2005). Biostatistics a practical approach to research and data handling. Mindex Publishing Company Limited, Ugbowo, Benin City, Edo State. pp. 169.

Ramesh, V.M. and Hilda, A. (1999). Incidence of keratinophilic fungi in the soil of primary schools and public parks of Madras City (India). Mycopathologia. 143(7): 139-145.

Rizwana, H.; Amal, A. and Siddiqui, I. (2012). Prevalence of Dermatophytes and other Keratinophilic fungi from soils of public parks and playgrounds of Riyadh, Saudi Arabia. The Journal of Animal and Plant Sciences. 22(4): 948- 953.

Sahoo, A.K. and Mahajan, R. (2016). Management of Tinea corporis, Tinea cruris, and Tinea pedis: A comprehensive review. Indian Dermatol Online Journal. 7(2): 77-86.

Sharma, R. and Rajak, R.C. (2003). Keratinophilic fungi: Nature's keratin degrading machines. 4th Edition. Their isolation, identification, and ecological role. Resonance. pp. 28-40.

Simpanya, M.F. and Baxter, M. (1996). Isolation of fungi from soil using keratin-baiting technique. Mycopathologia. 136(2): 85-89.

Singh, I.A.; Mishra, R. and Kushwaha, K. (2009). Dermatophytes, related keratinophilic and opportunistic fungi in indoor dust of houses and hospitals. Indian Journal of Medical Microbiology. 27 (7): 242-246.

Sunil, K.D. and Verekar, S.A. (2012). Prevalence of keratinophilic fungi in public park soils of Mumbai (India). Microbiology Research. 3(6): 12-22.

Vanbreuseghem, R. (1952). Biological technique for the isolation of dermatophytes from milk. Annals of the Belgian Society of Tropical Medicine. 32: 173-178.